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Validating in vivo Hyperpolarized ^{129}Xe Diffusion MRI and Diffusion Morphometry in the Mouse Lung

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Abstract

Purpose: Diffusion and lung morphometry imaging using hyperpolarized ^3He and ^{129}Xe are promising tools to quantify pulmonary microstructure noninvasively in humans and animal models. These techniques assume the motion encoded is exclusively diffusive gas displacement, but the impact of cardiac motion on measurements has never been explored. Furthermore, while diffusion morphometry has been validated against histology in humans and mice using ^3He , it has never been validated in mice for ^{129}Xe . Here, we examine the effect of cardiac motion on diffusion imaging and validate ^{129}Xe diffusion morphometry in mice.

Theory and Methods: One cohort of mice was imaged using respiratory-gated gradient-echo-based diffusion imaging, and ADC maps were generated with and without cardiac gating. Morphometric parameters were calculated from diffusion weighted images using Bayesian probability theory and compared against conventional histology.

Results: Cardiac gating had no significant impact on ADC measurements (dual-gating: $\text{ADC} = 0.020 \text{ cm}^2/\text{s}$, single-gating: $\text{ADC} = 0.020 \text{ cm}^2/\text{s}$, $p = 0.38$). Diffusion morphometry generated maps of ADC (mean $0.0165 \pm 0.0001 \text{ cm}^2/\text{s}$) and acinar dimensions ($h = 44 \mu\text{m}$, $R = 99 \mu\text{m}$, $L_m = 74 \mu\text{m}$) that correlated well with conventional histology ($h = 45 \mu\text{m}$, $R = 108 \mu\text{m}$, $L_m = 63 \mu\text{m}$).

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Conclusion: Cardiac motion has negligible impact on ^{129}Xe ADC measurements in mice, arguing its impact will be similarly minimal in humans where relative cardiac motion is reduced. Hyperpolarized ^{129}Xe diffusion morphometry accurately and non-invasively maps the dimensions of lung microstructure, suggesting it can quantify the pulmonary microstructure in mouse models of disease.

INTRODUCTION:

Hyperpolarized (HP) gas MRI using ^3He or ^{129}Xe enables non-invasive and radiation-free functional and structural lung imaging. The simplest use of HP gas MRI is to measure the distribution of gas within the lungs to provide an image of regional ventilation.¹ Beyond ventilation imaging, HP gas MRI can be combined with diffusion encoding to probe dimensions of the pulmonary microstructure. In small airspaces, particularly within the acinar spaces, diffusion is restricted by collisions with the alveolar walls, and this restriction results in an ADC that is significantly reduced. Because of this sensitivity to changes in microstructural size, HP gas ADC can be used to assess age-dependent increases in microstructural size due to normal growth and aging^{2,3} and due to pathological enlargement resulting in emphysematous tissue destruction in disorders like chronic obstructive pulmonary disease (COPD).^{4–8}

An important consideration in HP gas diffusion imaging is that bipolar diffusion gradients encode all motion within the ~10 ms diffusion encoding and acquisition window, and are not limited to detecting the diffusive, Brownian motion of HP gas atoms. Therefore, extraneous anatomical motion are expected to cause inaccuracies in the ADC measurement. Bulk and respiratory motion are mitigated by imaging during a breath-hold for human imaging or sedation, physical restraint, mechanical ventilation, and respiratory gating for animal imaging. However, mechanical coupling between the heart and lungs could drive coherent motion of the lung tissue and thus the gas residing therein, leading to spuriously high ADC. In particular, cardiac wall motion has been measured to be 1–3 mm throughout the cardiac cycle,⁹ which indicates this motion could have a significant impact on diffusion imaging. However, the effect of cardiac motion on ADC measurements has not yet been examined in either humans or animal models. Mice are an ideal model in which to examine the effect of cardiac motion, as they can be mechanically ventilated and imaged for longer periods than a human, their heart rate is 4- to 5-fold faster than a human, and their heart fills a large percentage of their thoracic cavity. That is, if cardiac motion effects ADC measurements, the impacts should be particularly visible in mice. Conversely, if cardiac motion has no effect on ADC measurements in mice, then there is a high degree of confidence that it will likewise have no effect on HP gas diffusion measurements in humans.

Using the same diffusion-encoding techniques, it is possible to directly estimate airspace dimensions by acquiring multiple different diffusion weighted-images and using an analytical model of the airspace geometry.^{10–14} These analysis methods, known as diffusion morphometry, use Bayesian probability theory alongside a simplified acinar model to calculate standard lung morphometry parameters such as mean linear intercept (L_m), surface to volume ratio (SV) and alveolar density (N_a) alongside detailed estimates of acinar dimensions including alveolar sleeve depth (h) and acinar duct radii (R). These quantitative

measures of lung microstructure are obtained non-invasively, thus allowing subtle changes in acinar morphology to be measured longitudinally during both normal development and disease progression.

Diffusion morphometry was developed in both preclinical and clinical settings primarily using HP ^3He MRI. As a part of this development, morphometric values calculated from ^3He images have been rigorously validated against direct morphometric measures using fixed specimens from human¹² and mouse lungs^{15–18}. Furthermore, ^3He diffusion morphometry has proven to be reproducible over time in healthy individuals¹⁹ and has shown sensitivity to emphysematous changes in the lungs.^{20,21} However, ^3He has become increasingly scarce and prohibitively expensive. As a result, the field of hyperpolarized gas MRI has moved almost universally to ^{129}Xe .

While ^{129}Xe is more affordable and has uniquely useful physical properties, such as the ability to dissolve in tissues and red blood cells,²² it historically generated much weaker signal intensity, because of its 3-fold lower magnetic moment and less mature polarization technology. However, recent advances in technology now routinely generate ^{129}Xe polarized of ~50% and ^{129}Xe -specific morphological models have been proposed.²³ Building on these advances, as has been done for ^3He , ^{129}Xe diffusion morphometry has now been well validated in humans, specifically by examining subjects with emphysematous disease both using explanted lungs^{24,25} and comparing to established ^3He morphometry.¹⁴ For preclinical imaging, ^{129}Xe diffusion morphometry has been demonstrated in rats,^{26,27} but has yet to be reported in mice. Because mice are the most widely used preclinical models of human lung disease, HP ^{129}Xe diffusion morphometry must be demonstrated and validated in mice before it can make a significant impact on basic and translational research.

In this work, we address two of the most significant potential limitations to preclinical HP gas diffusion imaging and diffusion morphometry using ^{129}Xe . Specifically, we examine the effect of cardiac motion on HP ^{129}Xe diffusion measurements in mice by acquiring diffusion weighted images with and without cardiac gating. Further, we report in vivo HP ^{129}Xe morphometry measurements in healthy mice and validate those results against ex vivo measurements made using conventional histology from excised lungs of the imaged mice.

THEORY:

Detailed descriptions of hyperpolarized gas diffusion morphometry in mice have been published previously,^{11,15,16,23,28} so we limit our discussion of theoretical background here. Briefly, gas diffusion is highly restricted by alveolar and airway walls. In mice, as with most mammals, the fundamental unit of the acinus is the alveolar sac, and this can be modeled by a network of cylindrical airways covered with alveolar sleeves (Figure 1).²⁹ The diffusion of gas within this geometry is anisotropic and can be characterized by longitudinal and transverse diffusion coefficients (D_L and D_T , respectively). Given the relatively coarse resolution of images, it is reasonable to assume a uniform distribution of airway distributions within a given voxel, so the MR signal can be calculated by²⁸

$$S = S_0 \exp(-bD_T) \left[\frac{\pi}{4bD_{an}} \right]^{1/2} \phi[(bD_{an})^{1/2}]. \quad (1)$$

In this equation, S_0 is the MR signal in the absence of diffusion weighting, $D_{an} = D_L - D_T$, ϕ is the error function, and b is a measure of the diffusion weighting applied. For our sequence, b is given by

$$b = (\gamma G_m)^2 \left[\delta^2 \left(\Delta - \frac{\delta}{3} \right) + \tau \left(\delta^2 - 2\Delta\delta + \Delta\tau - \frac{7}{6}\delta\tau + \frac{8}{15}\tau^2 \right) \right] \quad (2)$$

Here, γ is the gyromagnetic ratio of ^{129}Xe , G_m is the gradient amplitude, δ is the total width of each diffusion encoding gradient trapezoid, Δ is the time between the positive and negative diffusion encoding gradients, and τ is the ramp time.

Then, the relationships between diffusion coefficients and acinar geometric parameters are given by:²³

$$D_L = D_{L0}(1 - \beta_L \cdot bD_{L0})$$

$$D_T = D_{T0}(1 + \beta_T \cdot bD_{T0})$$

$$D_{L0} = D_0 \exp[-2.89 \cdot (1 - r/R)^{1.78}]$$

$$D_{T0} = D_0 \exp[-0.73 \cdot (L_2/R)^{1.4}] \cdot [1 + u(R, r)]$$

$$\beta_L = 35.6 \cdot (R/L_1)^{1.5} \exp(-4/\sqrt{1 - r/R})$$

$$\beta_T = 0.06 \quad (3)$$

$$L_1 = \sqrt{2D_0\Delta}$$

$$L_2 = \sqrt{4D_0\Delta}$$

$$u(R, r) = \exp[-A(R) \cdot (1 - r/R)^2] \cdot \left[\exp(-5(1 - r/R)^2) + 5(1 - r/R)^2 - 1 \right]$$

$$A(R) = 1.3 + 0.25 \cdot \exp\left[14 \cdot (R/L_2)^2\right]$$

Through numerical simulations, the dependence of DT and DL on the alveolar sleeve depth (h) and acinar duct radii (R) were determined, allowing h and R to be extracted from multi-b-value MRI experimental data. From these model parameters, remaining morphologic parameters can be calculated using

$$h = R - r$$

$$L = 2R \sin(\pi/8) = 0.765 R$$

$$\frac{S}{V} = \frac{2\pi R \cdot L + 2\pi(R^2 - r^2) + 16(R - r) \cdot L}{\pi R^2 \cdot L} \quad (4)$$

$$L_m = 4 \cdot V / S$$

$$N_a = 1 / (\pi R^2 \cdot L)$$

We also note that, when morphometry measurements are not desired, the apparent diffusion coefficient can be calculated simply using the diffusion equation either through fitting (for multi-b-value acquisition) or as an equation (for 2 b-value acquisition).

$$S = S_0 \exp(-b \cdot ADC) \quad (5)$$

METHODS:

Animals:

All procedures were approved by the Cincinnati Children's Hospital Medical Center's Institutional Animal Care and Use Committee. Two cohorts of animals were imaged, one to examine the effect of cardiac gating on diffusion measurements and one to validate HP ^{129}Xe morphometry. The Cardiac Gating Cohort comprised five healthy C57BL/6 Mice (3 female, 2 male; mean mass = 24.6 ± 4.5 g). Mice were anesthetized via intraperitoneal (IP) injection of triple sedative (67/3.3/0.17 mg/kg Ketamine/Xylazine/Acepromazine) and anesthesia was maintained with repeat doses of triple anesthetic (17/0.83/0.04 mg/kg) every 40–60 min as needed.

Electrocardiogram (ECG) leads (3M, St. Paul, MN) were secured on the front paws of each mouse and connected to an ECG module linked to monitoring software (Small

Animal Instruments (SAI), Inc, Stony Brook, NY). Throughout experiments, a small-animal physiological monitoring system (SAI, Inc Stony Brook, NY) was used to monitor and maintain body temperature via a thermocouple placed near the mouse.

Mice in this cohort were intubated with custom-built, metal-free cannulas that had a silicone cone at the end to ensure a tight seal with the trachea.^{30,31} The cannula was then connected via a Luer fitting (Cole Parmer, Vernon Hills IL) to a homebuilt, HP gas compatible ventilator.^{32,33} Mice were ventilated at a rate of 60 breaths per minute. The breath cycle was composed of a 200 ms inhalation, 500 ms breath hold, and 300 ms passive exhalation. When not imaging, mice were ventilated with a mixture of 25% O₂ and 75% N₂. During imaging, nitrogen was replaced with an equal volume of hyperpolarized xenon. The total volume of each breath was calculated based on the body weight of each animal (10 ml gas/kg mouse weight) and controlled by pressure regulators on the individual gas lines. The final pressure used to ventilate animals, as measured at the mouth of the animal, was 8–10 cm H₂O.

The Morphometry Cohort comprised eight C57BL/6 mice (4 female, 4 male) mean mass = 20.6 ± 2.1 g). Prior to MR experiments, mice were anesthetized by IP injection of 75-mg/kg sodium pentobarbital, and intubated with a 20-G catheter (NIPRO Medical Corp, Miami, FL) to provide an airtight seal for mechanical ventilation. Anesthesia was maintained as needed with periodic IP injections of sodium pentobarbital (25 mg/kg) which was chosen for its respiratory suppressing effects.³⁴

Animals were ventilated (70% N₂ and 30% O₂) at a rate of 100 breaths/minute. Tidal volume was adjusted such that the maximum inspiratory pressure for all animals was 10 cm H₂O, resulting in a tidal volume of ~0.25 ml. During ¹²⁹Xe MR acquisition, nitrogen was replaced with an equal volume of HP xenon and the breathing rate was reduced to 60 breaths/min (140-ms inhalation period and a 380-ms breath-hold, followed by passive exhalation) to provide a longer breath-hold for data acquisition.

Hyperpolarized ¹²⁹Xe Production and Delivery: Isotopically enriched (85% ¹²⁹Xe,) xenon was hyperpolarized to 20–55% polarization using a Polarean ¹²⁹Xe polarizer (Model 9810 or 9820, Polarean Inc., Durham, NC). HP ¹²⁹Xe was dispensed into 300-ml Tedlar bags (Jensen Inert Products, Coral Springs, FL) housed inside a pressurized cylinder (~3–6 psig). Prior to imaging, the Tedlar bag was placed in the fringe field of the 7T magnet at a location that generated a ¹²⁹Xe T₁ of ~45 min. Imaging was performed using a Bruker 7T Avance III horizontal, 30-cm bore, small animal MRI scanner.

Cardiac Gating Cohort

The coil used for the cardiac-gated diffusion measurements was a home-built quadrature bird cage coil with an inner diameter of 50 mm. Prior to animal imaging, the RF flip angle and approximate ¹²⁹Xe center frequency were calibrated on a pressurized ¹²⁹Xe phantom (~3 atm Xe, ~2 atm O₂). Animals were positioned with their lungs in the magnet isocenter by localizing with a 38 mm proton imaging coil (Bruker, Billerica, MA). Once the mouse was localized, the proton coil was replaced with the xenon coil, swapping coils from the rear of the magnet so that the animal could remain in place. For diffusion imaging, a custom 2D gradient-recalled echo (GRE) sequence with trapezoidal bipolar diffusion gradients was

employed (Figure 2). Sequence parameters included: orientation: axial, TR = 30 ms, TE = 4.8 ms, slice thickness = 1.5 mm, bandwidth = 50 kHz, field of view = 40×40 mm, matrix size = 64×64, flip angle = 45°, δ = 1.000 ms, τ = 1.010 ms, b-values = (0, 20) s/cm², number of averages = 4–8. The acquisition was iterated first by b then k increment. That is, all slices and b-values were acquired for a given phase encoding line before moving to the next phase encoding step. Averaging was performed by repeating whole-image-acquisition one after another. Both single slice and multi-slice acquisitions were used during imaging. For all acquisitions, it was ensured that at least one image slice went through the plane of the heart.

To enable data acquisitions that were timed to avoid respiratory motion, but that could be acquired in the presence or absence of cardiac motion, the sequence was designed to permit two triggering methods (Figure 2). In the first, the scanner was triggered only by the mechanical ventilator, and data acquisition began 50 ms into the 500-ms held inspiratory period (single-gating). In the second, cardiac gating was added to dual-gate for both respiratory and cardiac effects (dual-gating). To mitigate signal loss due to T₁ decay in the HP gas reservoir³⁵, which was ~ 45 minutes, all slices for given values of b and k were acquired in a single held breath when performing only respiratory gating. Only one slice with a given value of b and k was acquired with each cardiac trigger.

Morphometry Cohort

The coil used for the morphometry cohort was a home-built, nested ¹H/¹²⁹Xe (300.5/83.5 Mhz) quadrature birdcage transmitter/receiver with a 35mm inner diameter which provided a superior filling factor to the larger coil used for the cardiac gating cohort. Axial, diffusion-weighted MR images were acquired at end expiration using a multi-slice, gradient-echo sequence (see Fig. 2) as described above. Acquisition parameters included: matrix = 64×64, FOV = 32×32 mm², slice thickness = 1.5 mm, slices = 12, bandwidth = 50 kHz, dummy pulses = 9, α = 45°, TE = 7.95 ms, TR = 11.75 ms, δ = 2.575 ms, τ = 2.585 ms, b = (0, 6.25, 12.5, 18.75, 25, 31.25, 37.5) s/cm².

Image Analysis:

For the cardiac gating cohort, voxel-by-voxel ADC maps for both cardiac gated and non-cardiac-gated images were extracted from b = 0 and b = 20 images using equation 5. Masks for images were made from b = 0 images using a signal threshold (4 times the standard deviation of the noise) followed by an erosion and dilation with a spherical structuring element of radius 2. Manual intervention was used where necessary to improve image segmentation. Cardiac gated images were registered to non-cardiac-gated images using affine image registration tools in Matlab to compare regional ADC measurements.

For the Morphometry cohort, voxel-by-voxel maps of morphometric parameters were calculated from multi-b-value ¹²⁹Xe images using Bayesian probability theory,^{36,37} the acinar model of Weibel,³⁸ and the ¹²⁹Xe-specific theory of Sukstanskii and Yablonskiy,²³ which are summarized for small animals¹⁶ by Equations 1–4. Masks of images were generated from the b = 0 images using similar threshold-based segmentation as described above.

Histology: Following imaging, mice from the morphometry cohort were euthanized via exsanguination by opening the abdominal cavity and severing the abdominal aorta and inferior vena cava. The tracheal cannula was attached to a lung inflation apparatus, and the lungs were inflated with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) at a pressure of 10 cm H₂O to match *in vivo* ventilation conditions. The trachea was then secured with a silk suture and the tracheal cannula removed. Lungs were removed from the thoracic cavity and fixed in 4% PFA in PBS overnight. Fixed lungs were serially passaged to 70% ethanol, embedded in paraffin, and 5 μ m sections were then mounted on polysine slides and stained with hematoxylin and eosin. For one mouse, the lungs did not properly inflate during the fixing process, so morphometry was not performed for that individual.

Conventional morphometry was performed using a Nikon 90i inverted microscope (Nikon Instruments Inc., Melville, NY). Mean linear intercepts were determined using the methods of Dunnill, et al.³⁹ from thirty-five 10X imaging (5 from each of the middle, inferior and accessory lobes and 10 from right superior lobe and from the left lung.) Alveolar duct measurements were obtained using 4X tile scan images by visually identifying acinar duct structures transverse to the image plane. From each identified duct, 3 outer diameter measurements (2R) and six septal height measurements (h) were made. Shrinkage was accounted for, but was typically minimal.

Statistical Analysis:

Whole lung mean ADC measurements from cardiac-gated and non-cardiac-gated images were compared using Bland-Altman analysis and Wilcoxon signed-rank tests. Morphometric parameters acquired from ¹²⁹Xe morphometry were compared to histological metrics using Bland-Altman analysis and Wilcoxon signed-rank tests. Results were considered significant for $p < 0.05$.

RESULTS:

Cardiac Gating Cohort

Single and dual-gated images were successfully acquired from all 5 animals, with a total of 10 total single and dual-gated image pairs. The $b = 0$ images had a mean SNR of 8.97 ± 2.38 for single-gated images and 8.92 ± 2.27 for dual-gated images (Figure 3a, 3b). There were no obvious artifacts visible in either image set.

ADC maps were largely homogeneous (Figure 3c, 3d), and showed a relatively Gaussian distribution of ADC values for both types of gating (Figure 3e, 3f). Regional ADC features remained relatively consistent across both single- and dual-gated images (arrows). Importantly, there were no large or obvious differences near the heart between ADC maps for the two types of images. Difference maps between co-registered ADC maps were relatively homogeneous, with most values very close to zero (Figure 4a – 4c). For most images, the largest differences between the two ADC maps were located near large airways and the edges of the lungs. Histograms of voxel-by-voxel ADC difference showed an approximately Gaussian distribution centered about zero (Figure 4d).

Importantly, no systematic difference in ADC was observed in areas of the lung local to the heart. ROI selections of single-gated voxels in a representative mouse yielded ADC averages of $0.018 \pm 0.005 \text{ cm}^2/\text{s}$ away from the heart and $0.021 \pm 0.012 \text{ cm}^2/\text{s}$ near the heart. ROI selections of dual-gated voxels in the same subject yielded nearly identical ADC averages of $0.019 \pm 0.009 \text{ cm}^2/\text{s}$ away from the heart and $0.020 \pm 0.010 \text{ cm}^2/\text{s}$ near the heart.

Whole-image means of ADC across all images were nearly identical between the two types of gating, with single-gating images producing a mean ADC of $0.020 \pm 0.002 \text{ cm}^2/\text{s}$ and dual gating a mean ADC of $0.020 \pm 0.003 \text{ cm}^2/\text{s}$ (Table 1). Bland-Altman analysis (Figure 4e) showed that the mean difference between mean ADC measurements for both types of gating is very close to zero (mean difference $0.0003 \text{ cm}^2/\text{s}$). Further, the ADC difference between the two types of gating falls within the 95% confidence interval for all but one image. Thus, there is no systematic difference between whole-lung means using the two different gating methods. The overall difference between the whole-image means for the two types of gating was not significant ($p = 0.375$).

Morphometry Cohort

Diffusion-weighted images with adequate signal for diffusion morphometry ($\text{SNR} > 20$ on $b = 0$ images) were obtained from all mice. The mean whole-lung ADC for all mice in this cohort was $0.0165 \pm 0.0005 \text{ cm}^2/\text{s}$. Diffusion maps showed a relatively homogeneous pattern, as did maps of the subsequently-calculated morphometric parameters (Figure 5). Population means for alveolar sleeve depth, h , and acinar duct radius, R , were $44 \pm 1 \mu\text{m}$ and $99 \pm 2 \mu\text{m}$, respectively. From regional h and R measurements, additional morphometric parameters including mean linear intercept ($L_m = 74 \pm 3 \mu\text{m}$), alveolar number density ($N_a = 4600 \pm 200 \text{ mm}^{-3}$), and surface-to-volume ratio ($580 \pm 20 \text{ cm}^{-1}$) were calculated (Table 2). Histograms showed that voxel-by-voxel morphometric parameter values had relatively narrow, approximately Gaussian distributions (Figure 6). In particular, R and L_m had very narrow distributions, with most voxels falling within $\sim 10 \mu\text{m}$ of the mean value. The broadest distribution was that for alveolar number density, in which voxel values ranged from ~ 1800 to 7000 mm^{-3} .

Histology was performed on the fixed lungs of seven of the eight animals imaged in the morphometry cohort. In doing this, the population means for h and R were $45 \pm 1 \mu\text{m}$ and $108 \pm 3 \mu\text{m}$. The mean linear intercept for the cohort was $63 \pm 5 \mu\text{m}$. MRI-derived and histology-derived metrics were compared using Bland-Altman analysis. The mean difference between the two measures of alveolar sleeve depth is very near to zero ($-0.14 \mu\text{m}$) and all data-points fall within the 95% confidence interval (Figure 7a). The two measurements are not significantly different ($p = 0.83$). MRI measurements of acinar duct radius were measured to be $\sim 7 \mu\text{m}$ higher than histology measurements, and this difference is significant ($p = 0.03$) (Figure 7b). Even so, all but one animal's difference falls within the 95% confidence interval. The mean linear intercept measured by MRI was lower by $\sim 10 \mu\text{m}$ than that measured by histology (Figure 7c), and again the difference between the two measures is significant ($p = 0.02$). In this case, the difference for all animals falls within the 95% confidence intervals.

DISCUSSION:

Cardiac Gating Cohort

Based on the ADC measurements performed, the approximate diffusive motion of ^{129}Xe can be estimated. In particular, with a mean ADC of $0.02 \text{ cm}^2/\text{s}$ and δ of 1.0 ms, we can calculate the diffusion length to be $\sim 0.1 \text{ mm}$. Conversely, cardiac wall motion in mice is 1–3 mm,⁹ which suggests that cardiac motion could alter diffusion measurements significantly. However, HP ^{129}Xe diffusion measurements acquired both with and without cardiac gating showed ADC maps of similar magnitude and regional features. Furthermore, Bland Altman analysis showed that there were no systematic differences between the two measurements. Most importantly, there were no significant visible differences in ADC maps near the heart, where cardiac motion would be expected to have the greatest impact. In fact, difference maps between the two gating methods showed minimal difference between the two measurements in the majority of the lung volume. The largest differences appear at the periphery of the lungs and near large airways, which suggests that discrepancies are likely due simply to partial volume effects and registration imperfections rather than truly different ADC measurements. The lack of cardiac effect on ADC measurements can be understood by considering the timescale of the cardiac cycle. During imaging, mice typically had heart rates of ~ 200 beats per minute, corresponding to $\sim 300 \text{ ms}$ per cardiac cycle. In comparison, diffusion encoding occurs over $\sim 2 \text{ ms}$, suggesting that, despite relatively large tissue displacement near the heart, cardiac motion occurs over a long enough time scale that it has no impact on diffusion measurements.

These observations provide evidence that cardiac motion has a negligible impact on hyperpolarized ^{129}Xe diffusion imaging in mice and can thus be ignored in standard imaging protocols. This allows us to greatly simplify experimental setup for preclinical diffusion imaging. Most importantly, mice have a greater heart-to-lung volume ratio and a 10-fold higher heart rate than do humans. Thus, the absence of cardiac-induced changes to ADC values in mice strongly implies that cardiac motion can be safely ignored in human hyperpolarized ^{129}Xe diffusion measurements. Furthermore, because ^{129}Xe is 6-fold less diffusive than ^3He ,⁴⁰ its diffusion measurement is likely more sensitive to cardiac motion, these results indicate that cardiac motion can be ignored when performing HP ^3He diffusion imaging in both humans and animals.

Morphometry Cohort

Firstly, we note that the ADC values measured when using additional b-values were slightly lower ($\sim 0.016 \text{ cm}^2/\text{s}$) than those that were measured when performing comparisons between dual-gated and single-gated acquisition ($\sim 0.020 \text{ cm}^2/\text{s}$). Most likely, this is due to the smaller size of the animals imaged in the morphometry cohort (20.6 g vs 24.6 g).

Mean morphometric values acquired from diffusion imaging agreed well with those acquired via histology, particularly the measurement of alveolar sleeve depth. While the acinar duct radius was measured to be significantly lower using MRI morphometry, the values had an average difference from histological measures of less than 10%. Further, these values measured for h and R (h = $44 \mu\text{m}$, R = $99 \mu\text{m}$) closely agreed with previous measurements

in C57BL/6 mice using HP ^3He morphometry ($h = 51.7 \mu\text{m}$, $R = 97.2 \mu\text{m}$).^{16,17} The mean linear intercept measured using HP ^{129}Xe MRI was larger than that measured by histology by an average of 19% (MRI - $73 \mu\text{m}$, Histology - $63 \mu\text{m}$), which agrees quite well with the differences of 16% and 10% previously observed when using HP ^3He morphometry in mice (MRI - $60.5 \mu\text{m}$, Histology - $52 \mu\text{m}$), though we note that the pressures used in the helium study (15 cm H_2O) were higher than used here (10 cm H_2O).^{16,17}

The observed discrepancies between histological and MRI-derived morphometric parameters are unsurprising given the limitations of both HP gas morphometry and conventionally morphometry of explanted lungs. While HP gas imaging takes place in vivo, the acinar model used for calculating morphometric parameters could be a source of error in measurements. On the other hand, histology is performed using a non-physiological sustained inflation of the lungs prior to fixation, followed by staining and lung sectioning, all of which could distort the shape and dimensions of lung microstructure in ways that do not fully reflect in vivo structure. Ultimately, both methods of obtaining morphometric information have advantages and limitations, and while the agreement between the two is not perfect, the methods provide similar quantitative metrics of the pulmonary microstructure and it is impossible to say which is more representative of the true acinar structure in vivo. Most importantly, HP gas MRI enables longitudinal imaging of animals, where histology can only be performed as a terminal study.

Additional morphologic parameters calculated from diffusion weighted images included the alveolar number density and the surface to volume ratio over the whole lung. The alveolar number density measured in this work ($\sim 4600 \text{ mm}^{-3}$) is slightly higher than has been measured previously in healthy live C57BL/6 Mice using HP ^3He morphometry ($\sim 3800 \text{ mm}^{-3}$).^{16,17} However, the previous studies using HP ^3He morphometry have used a slightly higher peak ventilator pressure (15 cm H_2O) than used in the present study (10 cm H_2O), which would have led to a higher degree of lung inflation and thus a lower number density calculation. This possibility is supported by micro computed tomography (μCT) of ex vivo healthy C57BL/6 mouse lungs fixed using a pressure of 20 cm H_2O . Morphological analysis of these μCT images showed an alveolar number density of $\sim 3000 \text{ mm}^{-3}$.^{41,42} Interestingly, conventional morphometry yields an alveolar number density about twice that obtained by these imaging methods ($9550, 7000\text{--}9000 \text{ mm}^{-3}$)^{43,44}, which highlights the potential for inherent differences between conventional and imaging-based methods and conventional ex vivo morphometric parameters.

The surface to volume ratio in this work (580 cm^{-1}) agrees well with previous measurements in the same strain of mice. For example, in vivo ^3He morphometry yields a surface to volume ratio of 698 and 670 cm^{-1} .^{15,16} Measurements in explanted lungs have computed similar values of 640, 600, and 520 cm^{-1} .^{41,43,44} The close agreement of mean ^{129}Xe MRI morphometry metrics with histological measurement and values found in the literature provides confidence that HP ^{129}Xe diffusion imaging is capable of providing accurate morphometric values.

To this point, we have discussed our ^{129}Xe morphometry results in the context of whole-lung means. However, this method of quantifying lung morphology is able to provide maps

of the relevant morphometric parameters regionally over the whole lungs. The maps of h , R , L_m , N_a , and SV produced show a high degree of homogeneity over the whole lung, which is expected in healthy mice and matches the results observed in previous ^3He morphometry studies.^{16,17} These homogeneous maps (Figure 5) and associated narrow histograms (Figure 6), alongside good agreement with histology (Figure 7) and previous morphometry results both in vivo and ex vivo indicate that hyperpolarized ^{129}Xe can be used effectively for diffusion morphometry, even in the small volumes (0.25 mL) being imaged in mouse lungs.

Conclusions:

Imaging diffusive motion of hyperpolarized gas has become an effective way of non-invasively probing pulmonary airspace dimensions. In this work, we have performed diffusion imaging using HP ^{129}Xe in mice, examining the effect of cardiac motion on ADC measurements and performing diffusion morphometry. It was found that cardiac motion does not affect ADC measurements, providing confidence that HP gas ADC measurements, and by extension diffusion morphometry, are robust to cardiac motion both for preclinical and human imaging. Hyperpolarized ^{129}Xe diffusion morphometry measurements agreed well with conventional histology and with previous morphometry measurements using ^3He in the same mouse strain. Ultimately, our results show that HP ^{129}Xe diffusion morphometry can be effectively used for probing mouse lung airspace dimensions.

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Figure 1.

Geometric model used for diffusion morphometry. (a) Model of the acinar duct used as a theoretical model of lung microstructure. (b) Face-on view of geometric model, specifying R and h . (c) Lung histology from a C57BL/6 mouse showing an acinar duct, demonstrating the biological relevance of the geometric model.

Figure 2.

(a) Modified gradient echo sequence used for HP ^{129}Xe diffusion weighted imaging sequence. (b) Schematic showing respiratory and cardiac gating methods, with cardiac trigger windows shown in the top row, respiratory trigger windows shown in the middle row, and the combined trigger windows shown in the bottom row.

Figure 3.

ADC Measurement for both respiratory and respiratory + cardiac gated acquisition. Representative single (a) and dual-gated $b=0$ images. ADC maps corresponding to these images for single (a) and dual-gated (b) acquisition. Similar regional ADC patterns in both images are highlighted by arrows. Histograms of voxel-by-voxel ADC for single (e) and dual-gated (f) ADC maps show a relatively Gaussian pattern.

Figure 4.

Regional ADC difference mapping. Representative ADC map slices are shown for both single (a) and dual-gated (b) acquisition. Prior to ADC map generation, dual gated images were registered to single gated images. (c) Voxel-by-voxel difference maps showing dual-gated – single-gated ADC. (d) Histogram of difference voxels shows a relatively Gaussian distribution centered around zero. (e) Bland-Altman analysis shows no systematic difference (mean 0.0003 cm²/s) between the whole-lung ADC measured using different gating methods.

Figure 5.

Representative HP ^{129}Xe morphometry maps. Rows from top to bottom show: Magnitude image ($b = 0$), ADC maps (whole-lung mean: $0.016 \pm 0.004 \text{ cm}^2/\text{s}$); Alveolar sleeve depth (whole-lung mean: $44 \pm 8 \text{ }\mu\text{m}$), Acinar duct radius (whole-lung mean: $98 \pm 13 \text{ }\mu\text{m}$), Mean linear intercept (whole-lung mean: $73 \pm 22 \text{ }\mu\text{m}$), Alveolar number density (whole-lung mean: $4300 \pm 200 \text{ mm}^{-3}$), and Surface-to-Volume ratio (whole-lung mean: $590 \pm 150 \text{ cm}^{-1}$)

Figure 6.

Histograms showing distributions of metrics obtained from ^{129}Xe MRI morphometry for a representative mouse. (a) ADC, (b) alveolar sleeve depth, (c) acinar duct radius, (d) mean linear intercept, (e) alveolar number density, (f) Surface-to-Volume ratio. In all cases, histograms have narrow distributions and relatively Gaussian profiles.

Figure 7.

Bland-Altman analysis comparing histology to ^{129}Xe morphometry. (a) There is no systematic difference between measurements of alveolar sleeve depth. However, MRI morphometry slightly underestimates acinar duct radius (b) and slightly overestimates mean linear intercept (c) as compared to histology.

Table 1:

Cardiac-gating ADC measurements summary. Values are mean $\pm$ standard deviation.

Mouse	Mass	Number of Slices	Mean Single-Gated ADC ($\times 10^{-2}$ cm ² /s)	Mean Dual-Gated ADC ($\times 10^{-2}$ cm ² /s)	Difference ($\times 10^{-2}$ cm ² /s)
1	25	3	2.0 $\pm$ 1.7	2.1 $\pm$ 1.4	-0.1
		1	2.1 $\pm$ 1.5	2.1 $\pm$ 0.9	0.1
2	22	1	1.8 $\pm$ 1.5	2.0 $\pm$ 1.4	-0.1
		5	2.0 $\pm$ 1.4	2.1 $\pm$ 1.4	-0.1
		5	2.2 $\pm$ 1.3	2.3 $\pm$ 1.7	-0.1
3	19.5	5	1.9 $\pm$ 1.5	2.2 $\pm$ 1.3	-0.2
		1	2.2 $\pm$ 1.6	2.3 $\pm$ 1.7	-0.1
4	31.5	5	2.4 $\pm$ 2.0	2.3 $\pm$ 1.9	0.1
5	25	1	1.8 $\pm$ 1.0	1.8 $\pm$ 1.1	0.0
		5	1.7 $\pm$ 1.5	1.3 $\pm$ 1.2	0.4
Population	24.6 $\pm$ 4.5		2.0 $\pm$ 0.2	2.0 $\pm$ 0.3	-0.01

Table 2:

Morphometry summary. Values are mean $\pm$ standard deviation.

Mouse	Mass g	HP ^{129}Xe Diffusion Morphometry						Histology		
		ADC $\times 10^{-2} \text{ cm}^2/\text{s}$	h μm	R μm	Lm μm	Na mm^{-3}	SV cm^{-1}	h μm	R μm	Lm μm
1	24	1.61 $\pm$ 0.03	45 $\pm$ 7	99 $\pm$ 10	73 $\pm$ 18	4500 $\pm$ 1200	580 $\pm$ 120	44 $\pm$ 9	106 $\pm$ 8	56 $\pm$ 9
2	22	1.67 $\pm$ 0.04	44 $\pm$ 7	100 $\pm$ 11	75 $\pm$ 20	4500 $\pm$ 1400	570 $\pm$ 140	44 $\pm$ 9	107 $\pm$ 16	69 $\pm$ 12
3	22	1.64 $\pm$ 0.04	44 $\pm$ 8	99 $\pm$ 13	74 $\pm$ 22	4700 $\pm$ 1500	590 $\pm$ 150	43 $\pm$ 9	112 $\pm$ 17	62 $\pm$ 7
4	19	1.58 $\pm$ 0.04	44 $\pm$ 8	96 $\pm$ 13	70 $\pm$ 21	5000 $\pm$ 1700	620 $\pm$ 160	46 $\pm$ 9	102 $\pm$ 13	69 $\pm$ 12
5	19	1.74 $\pm$ 0.05	43 $\pm$ 7	102 $\pm$ 16	80 $\pm$ 28	4400 $\pm$ 1700	560 $\pm$ 170	46 $\pm$ 11	111 $\pm$ 17	59 $\pm$ 12
6	18	1.71 $\pm$ 0.04	43 $\pm$ 8	101 $\pm$ 15	78 $\pm$ 26	4500 $\pm$ 1600	560 $\pm$ 160	45 $\pm$ 8	111 $\pm$ 16	65 $\pm$ 8
7	22	1.60 $\pm$ 0.04	44 $\pm$ 7	97 $\pm$ 12	71 $\pm$ 20	4900 $\pm$ 1500	600 $\pm$ 150	40 $\pm$ 8	93 $\pm$ 19	61 $\pm$ 10
8	19	1.62 $\pm$ 0.03	45 $\pm$ 7	99 $\pm$ 10	73 $\pm$ 18	4500 $\pm$ 1100	570 $\pm$ 120	--	--	--
Population	20.6 $\pm$ 2.1	1.65 $\pm$ 0.01	44 $\pm$ 1	99 $\pm$ 2	74 $\pm$ 4	4600 $\pm$ 240	580 $\pm$ 23	45 $\pm$ 1	108 $\pm$ 3	63 $\pm$ 5